Minimising the development of anthelmintic resistance, and optimising the use of the novel anthelmintic monepantel, for the sustainable control of nematode parasites in Australian sheep grazing systems.

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Abbreviations list

AAD	Amino-acetonitrile derivative
ABA	Abamectin — non-persistent ML
BZLV	Combination treatment of BZ+LEV
BZ	White drenches or benzimidazoles — e.g. albendazole, oxfendazole
COM	Combination treatment of BZ+LEV+ABA
LEV	Clear drenches or imidazothiazoles — levamisole
ML	Macrocyclic lactones — abamectin, doramectin, ivermectin, moxidectin
MOX	Moxidectin — persistent ML
MPL	Monepantel (an AAD)
NSW	New South Wales
VIC	Victoria
WA	Western Australia
WB	Worm (nematode) burden

Abstract

Objective To compare the risk of different treatment scenarios on selecting for anthelmintic resistance on Australian sheep farms.

Design A computer simulation model predicted populations of *Trichostrongylus colubriformis*, *Haemonchus contortus* or *Teladorsagia* (*Ostertagia*) *circumcincta*, and the frequency of anthelmintic resistance genes.

Method Nematode populations and the progression of drug resistance for a variety of treatment options and management practices in sheep rearing areas of WA, VIC and NSW were simulated. A scoring system was devised to measure the success of each option in delaying resistance to each anthelmintic and in controlling nematode populations.

Results The best option at all sites was combining the new anthelmintic (monepantel) with a triple mixture of benzimidazole, levamisole and abamectin (COM). The next best

option was: in NSW, rotation at each treatment between monepantel, moxidectin and COM; in VIC, rotation at each treatment between monepantel and COM; and in WA, rotation at each treatment between monepantel (used in winter) and COM or moxidectin (used in summer-autumn). In WA, rapid selection for resistance occurred as a consequence of summer-autumn treatments; however, if a small percentage of adult stock were left untreated then this selection could be greatly reduced. Despite purposely assuming relatively high resistance to benzimidazole and levamisole, COM was still effective in controlling worms and delaying resistance.

Conclusions Due to cost constraints, it may not be feasible or profitable for producers to always use the combination of all drugs. However, the second and third best options still considerably slowed the development of anthelmintic resistance.

Key words: anthelmintic resistance, monepantel, grazing management, sheep, nematode control, simulation model

Introduction

The release of the first compound from the new amino-acetonitrile derivative (AAD) anthelmintics in New Zealand[1,2] (monepantel, MPL; Zolvix®, Novartis Animal Health Inc., Switzerland) is an opportune time to examine how best to use a new class of anthelmintics on Australian sheep farms. Important considerations include both the rate of development of anthelmintic resistance and the effectiveness of control options in reducing the production losses from nematodes. Laboratory and field studies can contribute useful information, but they take many years to conduct and are expensive. Consequently, the only practical way to achieve this analysis in the short-term is by using computer models that simulate populations of gastro-intestinal parasites in grazing ruminants.[3] Generally models[4-6] simulate either mono-specific or 'general' nematode[7] infections rather than multiple parasite species. The complexity resulting from combining a number of single species models may only add to an overload of information, without adding to the understanding gained from simply using the models individually. To simplify interpretation from a multi-species model we developed a single measure, for a control option, which reflects the development of drug resistance to all broad-spectrum anthelmintic classes as well as the ability to control the major nematode species at a particular site.

Nematode control regimens used in the main sheep farming areas of Australia were modelled and a variety of drug use options were examined for each area. The regimens modelled were relatively prescriptive systems; Barger[8] previously pointed out that before implementing a control option, farmers will consider effectiveness, cost and ease of applying the strategy, with the 'sustainability' of an option likely to be a lower order priority. Thus, as more complex systems are less likely to be maintained by producers, relatively simple and straightforward regimens, considered likely to impede the development of drug resistance were tested. While routine faecal worm egg count monitoring to optimise treatment times are recommended, it has been adopted by only a minority of sheep producers to date.

A simple sustainable way to delay selection for anthelmintic resistance is to apply a combination treatment of effective unrelated drugs.[3,6,9-12] This is not always possible because resistance to the current anthelmintics may be present on a farm, or there is a reluctance by producers to use multi-active treatments when a single active is still effective or cheaper.

Therefore, the use of a new drug class (in this case, the AADs represented by MPL) in combination with existing drugs with a relatively high level of resistance (i.e. benzimidazole [BZ] and levamisole [LEV]) or moderate level of resistance (i.e. abamectin [ABA]), and the rotation of the new drug with other anthelmintics (e.g. moxidectin [MOX] and COM; where COM is a mixture of BZ+LEV+ABA), was studied. By exploring the potential outcomes of these options for all anthelmintics sound advice will be available to graziers; certain options may involve sacrificing some of MPL lifespan to prolong the useful life of other anthelmintics.

When a new anthelmintic class is released, the expected resistance (R)-allele frequency is likely to be very low, say one in a million, and resistance would take a relatively long time to develop. Additionally, the genetics of inheritance for resistance are unknown. In this study, the initial MPL R-allele frequency was purposely set at a level higher than that expected for a new drug so that resistance to MPL would artificially develop in a relatively short time when used alone. Resistance was also assumed to be co-dominant so that resistance would develop in a shorter period than if resistance was a recessive trait. With these measures, the options that delayed resistance could be more readily identified by computer simulation. Monepantel here is representative of any new drug and the simulations can be refined when information about MPL resistance is obtained; however, it is important that various drug management options are explored before resistance emerges.

Materials and Methods

Local weather data and management practices for self-replacing Merino flocks at Kojonup (Western Australia, WA), Hamilton (Victoria, VIC) and Glen Innes (New South Wales, NSW) were used to simulate: *T. circumcincta* and *T. colubriformis* populations at the first two sites, and *H. contortus* and *T. colubriformis* populations at the NSW site. The assumptions required to simulate the evolution of anthelmintic resistance are described in detail in Appendix 1. Monepantel and macrocyclic lactone (ML) resistance were simulated independently. However, because the model[5] can only simulate resistance to three drugs simultaneously, resistance to BZ and LEV was modelled as a single drug given as a combination (denoted as BZLV, Appendix 1). The initial R-allele frequency for MPL was 0.001% for *Trichostrongylus* and *Haemonchus*, and 0.003% for *Teladorsagia*. The R-allele frequency for MPL was set such that MPL resistance developed in no more than 10 years when MPL was used exclusively under the conditions simulated. Resistance to ML was assumed to be common, and to BZLV very common, and so the R-allele frequencies for ML and BZLV were set at 3% and 40%, for all species, respectively. The models described by Barnes and Dobson[5] for *T. colubriformis* and Roberts and Swan[4] for *H. contortus* were modified to predict single or concurrent populations of *T. circumcincta*, *T. colubriformis* and *H. contortus* in sheep and on pasture.[13] All simulations were run for 20 years.

Treatment options simulated

The following four options were simulated at all sites: 1. MPL+COM; 2. MPL/COM rotation; 3. MPL/MOX rotation; 4. MPL/COM/MOX rotation. The effect of changing the sequence of treatments within a rotation was also examined for options 2-4 in WA. For option 1, at each scheduled anthelmintic treatment a combination of MPL and COM was applied. Drug rotations (options 2-4) were within the annual treatment cycle and not based on a calendar year (Tables 1-3). Four ‘control’ treatments were also simulated, against which the

effectiveness of the four treatment options to reduce nematode populations and select for drug resistance could be measured. The control treatments were: Untreated; MPL only; COM only; and MOX only.

Simulations of all the drug treatment options were then repeated, but with 1 to 10% of adult stock not receiving any anthelmintic treatment. All lambs received their scheduled treatments in these simulations. This was performed to assess the effects of leaving some nematodes not exposed to anthelmintics on selection for drug resistance and effectiveness of parasite control. The details of this analysis are reported with the description of the multi-species model[13] but are briefly discussed in the results because refugium is a key issue, particularly in WA.

'Summer-autumn drenching' in Western Australia

The management schedule is set out in Table 1. The ewes were not given any anthelmintic treatment while on paddock 3, with the aim to provide a relatively unselected nematode population (i.e. 'refugia') for lambs that grazed this paddock in May. The drug sequence used for the rotation options (2-4) are shown in Table 1. Additional simulations for options 2-4 were run where a single MPL treatment was given to ewes in April and to lambs in December (options 2a-4a), and the alternate drugs were used for the other treatments. This was done to evaluate the impact of changing the drug sequence in an environment with a harsh, dry summer, such as Kojonup.

Table 1 Basic management and anthelmintic treatments applied to ewes and lambs in WA.

			OPTIONS:					
EWES	WEEK	ACTION	2	3	[a]4	2a	3a	[a]4a
Early Jul	1	Lambing on paddock 1						
Late Dec	25	Move to paddock 3						
Early Apr	40	Drench move to paddock 1	COM	MOX	[a]M/C	MPL	MPL	MPL
Early Jun	49	Drench	MPL	MPL	MPL	COM	MOX	[a]M/C
LAMBS	AGE	ACTION						
Early Jul	1	Born on paddock 1						
Early Oct	14	Drench move to paddock 2	MPL	MPL	COM	COM	MOX	COM
Late Dec	25	Drench	COM	MOX	MOX	MPL	MPL	MPL
Early May	44	Move to paddock 3						
Early Jun	48	Drench	MPL	MPL	MPL	COM	MOX	MOX
Late Jun	52	Remove from system						

[a] M/C treatment with MOX or COM to ewes was alternated annually for option 4 and 4a.

'Wormplan' in Victoria

The management schedule and sequence of drug use for options 2-4 in VIC are shown in Table 2. In late October, dry adult stock that grazed paddock 2 (not shown in Table 2) were moved to paddock 3, three weeks before lambs were weaned onto paddock 2. In early February, they were interchanged with the weaners on paddock 2. The dry adults were drenched in mid-November and early February. Note for option 4, the dry adults were not given a COM treatment as they only received two treatments per annum, and it was assumed they would receive the same anthelmintic when the ewes were treated.

Table 2 Basic management and anthelmintic treatments applied to ewes and lambs in VIC.

EWES	WEEK	ACTION	Option 2	Option 3	Option 4
Mid Aug	1	Lambing on paddock 1			
Mid Nov	13	Drench (weaning)	COM	MOX	MOX
Early Feb	24	Drench	MPL	MPL	MPL
Late Jul	49	Drench (pre-lamb)	COM	MOX	COM
LAMBS	AGE	ACTION			
Mid Aug	1	Born on paddock 1			
Mid Nov	13	Drench and move to paddock 2	COM	MOX	MOX
Early Feb	24	Drench and move to paddock 3	MPL	MPL	MPL
Late May	41	Drench	COM	MOX	COM
Mid Aug	52	Remove from system			

'Worm Kill' in New South Wales

The basic treatment schedule for options 2-4 is mainly for short-acting anthelmintics (Table 3). For the MOX only control regimen, ewes received pre-lambing, weaning, mid-February and mid-April treatments; lambs received late October, mid-December, mid-February, mid-April and late August treatments. More treatments are given here than in WA or VIC because of the risk posed by *H. contortus*.

Table 3 Basic management and worm control operations applied to ewes and lambs in NSW.

EWES	WEEK	ACTION	Option 2	Option 3	Option 4
Mid Sep	1	Lambing on paddock 1			
Mid Dec	13	Drench (weaning)	MPL	MPL	MPL
Early Feb	20	Drench	COM	MOX	MOX
Mid Mar	26	Drench	MPL	*nt*	COM
Late Apr	33	Drench	COM	MPL	MPL
Late Aug	49	Drench (pre-lamb)	MPL	MOX	MOX
LAMBS	AGE	ACTION			
Mid Sep	1	Born on paddock 1			
Late Oct	7	Drench	COM	MOX	COM
Mid Dec	13	Drench and move to paddock 2	MPL	MPL	MPL
Early Feb	20	Drench	COM	MOX	MOX
Mid Mar	26	Drench	MPL	MPL	COM
Late Apr	33	Drench	COM	MOX	MPL
Late Jun	41	Drench	*nt*	*nt*	MOX
Late Aug	49	Drench	MPL	*nt*	*nt*
Mid Sep	52	Remove from system			

nt = no treatment.

Estimating host mortalities

See Dobson et al.[13] for a full description of how deaths from concurrent nematode infections were estimated; a brief summary follows. Lethal parasite burdens in lambs and adult sheep were assumed to be 50,000, 25,000 and 15,000 for *T. colubriformis*, *T. circumcincta* and *H. contortus*, respectively (except lethal *H. contortus* burden for lambs was set at 10,000).

Predicted *T. circumcincta* and *H. contortus* worm burdens were converted to *T. colubriformis* 'equivalents'. For example, in lambs total *T. colubriformis* equivalents was:
T. colubriformis equivalents = *T. colubriformis* + 2 x *T. circumcincta* + 5 x *H. contortus.*
The factors used above to convert *T. circumcincta* (2) and *H. contortus* (5) to *T. colubriformis* are: (*T. colubriformis* lethal burden/*T. circumcincta* lethal burden), and (*T. colubriformis* lethal burden/*H. contortus* lethal burden), respectively. The predicted nematode burden and dispersion parameter (k) of the negative binomial distribution[5] were used to estimate the proportion of animals with *T. colubriformis* equivalents greater than the *T. colubriformis* lethal nematode burden. This proportion of the flock was assumed to die. The mean nematode burdens of each species were then adjusted to account for the loss of worms in the heaviest infected animals.

Ranking the relative effectiveness of the options
Two scores were formulated to rate each option on their ability to (a) delay selection for resistance, and (b) control nematodes. Scores were then pooled to (c) determine ranks. To derive these scores, weights, based on expert opinion[14] were assigned to scale the importance of mean nematode (worm) burden (WB), sheep deaths and anthelmintic resistance. Weights ranged from 1 = unimportant to 5 = very serious.

(a) For resistance to MPL, ML and BZLV the weights 5, 4.2 and 2.5 were used, respectively. To determine the resistance score for each species and option:
(1) The relative time (RT) to resistance for each drug class (MPL, ML and BZLV) was obtained by dividing the time to reach 50% R-alleles by the time to 50% R-alleles when resistance developed fastest, e.g. a RT of two for a particular option and drug indicates it took twice as long for resistance to develop.
(2) The RT was converted to a mark from 0 to 100 such that the RT of 1 (fastest time to resistance) became 100 and was 0 if 50% R-alleles was not reached in 20 years.
(3) The mark was then multiplied by the weight (see (a) above) for each drug class, i.e. the maximum mark for MPL, ML and BZLV was 500, 420 and 250, respectively.
(4) The average of the weighted mark was the resistance score for the nematode species and option; the higher the score the more rapidly resistance developed.

(b) For the nematode control score, weights of 3.9, 5.0, 3.7 and 3.6 were used for ewe WB, ewe death, lamb WB and lamb death, respectively. In addition, weights of 4.5 and 3.5 were used for means over years 1-6 and 1-20, respectively. For each species and option the nematode control score was determined by:
(1) Converting the ewe and lamb mean WB and death rates for years 1-6 and 1-20 to a percentage of the maximum mean WB and death rate (from the untreated control), i.e. provides eight values (2 animal classes * 2 measures * 2 periods).
(2) The weighted sum (over ewe and lamb using above weights) of percentage WB and death rate for years 1-6 and 1-20 was tallied to yield four values.
(3) The mean of these four values weighted for years was the nematode control score for the nematode species and option; the higher the score the worse nematode control was.

(c) *Pooling the scores to determine ranks*. In each state, species were assigned different weights: for *T. colubriformis* being 4.2, 4.8 and 4.6 in WA, VIC and NSW, respectively; *T. circumcincta* weights were 4.6 in WA and 4.2 in VIC; and in NSW, *H. contortus* was 4.6. The weighted average over the two species for each option in each state was then determined. The

total score was the sum of the pooled nematode control and resistance score. Ranks within each state were then determined for the options (Tables 4-6).

In summary: The 'total score' and 'rank' rates the options on their ability to control nematodes and delay resistance expressed as a single measure. Tables 4-6 also provide results expressed as percentage effectiveness to control nematodes and to delay resistance. 'Effectiveness of nematode control' is the death rate plus nematode burden score for each option expressed as a percentage reduction of the score for the untreated controls, which is equivalent to 'efficacy'. 'Effectiveness to delay resistance' is the resistance score for each option expressed as a percentage reduction from the highest resistance score from the treatment controls where each drug was used exclusively.

Results

The 'total score' (in Tables 4-6) provides a composite estimate of a particular option's ability to control nematodes and delay resistance. From Tables 4-6, option 1 (MPL+COM) consistently ranked best because of its superior ability to delay selection for resistance, though some of the MOX-rotation treatments showed better worm control (option 4 in NSW and options 3 and 4 in VIC). Option 1 is excluded from the site-specific reporting of the results below.

Western Australia
When used solely, resistance to MPL did not develop if the R-allele frequency was less than 4 and 9 per 10^6 for *T. colubriformis* and *T. circumcincta*, respectively. The sequence of drugs used for options 2-4 was important; Table 4 gives the results for winter (options 2-4) and summer/autumn use of MPL (options 2a-4a). It is clear from this table that it was preferable to use a new anthelmintic in winter (i.e. the sequence given for option 2-4 in Table 1) and then use COM or MOX at other times, but this generally led to more rapid selection of resistance to the drug used in summer or autumn (i.e. when there was little refugia from anthelmintics in the external environment). This could be overcome by leaving 1-4% of adult ewes untreated (data not shown); the MPL/COM option effectiveness to delay resistance rose from 51% to 100% while only declining slightly in its effectiveness to control parasites. More detailed analysis of this strategy is published elsewhere.[13]

'Wormplan' in Victoria
When used solely, resistance to MPL did not develop if the R-allele frequency was less than 1 and 10 per 10^6 for *T. colubriformis* and *T. circumcincta*, respectively. Here option 2 (MPL/COM) ranked best and was comparable to MPL+COM. Unlike WA, its ability to delay selection for drug resistance was not enhanced even when 10% of adults were left untreated.[13] Using MPL as the first rather than the second summer treatment had little effect on the outcome (data not shown).

'Worm Kill' in New South Wales
When used solely, resistance to MPL did not develop if the R-allele frequency was less than 2 and 5 per 10^6 for *T. colubriformis* and *H. contortus*, respectively. Here option 4 (MPL/COM/MOX) was the next best option after MPL+COM (Table 6). In the untreated control simulation the average deaths per year was 8% (range 2 to 20%) and attributable in

approximately equal measure to both *T. colubriformis* and *H. contortus*. For years 1-6, i.e. prior to resistance fully emerging, mean deaths for the four treatment options and treated controls was 1.5% (range between years 0.7 to 2.6%) and regimens using either short- or long-acting anthelmintics were effective.

Additional simulations were run in an environment where *H. contortus* was a more serious threat.[13] In this situation, only *H. contortus* was simulated and deaths in untreated controls increased to 28% (range 2 to 81%). For years 1-6, treatment options and controls that included MOX reduced deaths to a mean of 5.5% and the MPL/MOX rotation option yielded the lowest death rate of 1.1%. For options and controls that only used short-acting anthelmintics the death rate in years 1-6 was reduced to 13.9% as the treatment schedule (Table 3) provided insufficient short-acting treatments or grazing management to effectively control *H. contortus* in this environment.

Table 4 The effect of seven treatment options simulated in impeding the development of anthelmintic resistance and controlling *T. colubriformis* and *T. circumcincta* in WA.

% Effectiveness of worm control	% Effectiveness to delay resistance	Death rate %[c]	Mean worm score[a]	Mean resistance score[a]	Total score	Score rank	Treatment options[b]: MPL given in winter
94	100	0.1	40	0	40	1	1.MPL+COM
91	51	1.2	54	82	136	2	2.MPL/COM
92	46	1.5	48	91	139	3	3.MPL/MOX
93	17	0.5	44	138	182	4	4.MPL/COM/MOX
							MPL use: summer/autumn
83	19	9.9	102	136	238	5	4a.MPL/COM/MOX
82	21	9.9	109	131	240	6	2a.MPL/COM
83	11	9.8	102	149	250	7	3a.MPL/MOX
Highest scores from controls			614	167			

[a]Unlike % effectiveness, the lower the score the lower the resistance level and worm populations. Resistance score is a weighted mean of MPL, ML and BZLV R-alleles. The worm score includes host death and worm burden and is a weighted mean of the two species involved (see text); [b]Options are ranked over seven treatments options (i.e. including winter and summer/autumn MPL use); [c]Mean death rate for lambs and ewes.

Table 5. The effect of four treatment options simulated in impeding the development of anthelmintic resistance and controlling *T. colubriformis* and *T. circumcincta* in VIC.

% Effectiveness of worm control	% Effectiveness to delay resistance	Death rate %[c]	Mean worm score[a]	Mean resistance score[a]	Total score	Score rank	Treatment options
93	100	0.4	30	0	30	1	1.MPL+COM
93	100	0.4	30	0	30	2	2.MPL/COM
94	83	0.2	27	29	56	3	4.MPL/COM/MOX
94	78	0.2	26	36	63	4	3.MPL/MOX
Highest scores from controls			419	167			

[a]Unlike % effectiveness, the lower the score the lower the resistance level and worm populations. Resistance score is a weighted mean of MPL, ML and BZLV R-alleles. The

worm score includes host death and worm burden and is a weighted mean of the two species involved (see text); [c]Mean death rate for lambs and ewes.

Table 6 The effect of four treatment options simulated in impeding the development of anthelmintic resistance and controlling *T. colubriformis* and *H. contortus* in NSW.

% Effectiveness of worm control	% Effectiveness to delay resistance	Death rate %[c]	Mean Worm score[a]	Mean resistance score[a]	Total score	Score rank	Treatment options
77	100	1.8	116	0	116	1	1.MPL+COM
83	52	0.9	87	81	168	2	4.MPL/COM/MOX
68	68	2.6	162	54	216	3	2.MPL/COM
56	58	3.7	223	70	293	4	3.MPL/MOX
Highest scores from controls			509	167			

[a]Unlike % effectiveness, the lower the score the lower the resistance level and worm populations. Resistance score is a weighted mean of MPL, ML and BZLV R-alleles. The worm score includes host death and worm burden and is a weighted mean of the two species involved (see text); [c]Mean death rate for lambs and ewes.

Discussion

Rotations between anthelmintic classes were within the annual sheep production system because this allows for greater flexibility than calendar-based (i.e. year-to-year) drench rotations. In some circumstances a drug with persistent activity may be required, while at most times a short-acting high efficacy treatment is the preferred option.[9] Rotating between drug classes on an annual calendar basis does not allow for this kind of flexibility.

A key result was that a combination of all anthelmintic classes, including MPL, was the best option for delaying the development of anthelmintic resistance while achieving effective worm control. However, because of cost it is unlikely to be routinely applied for all 'strategic' or 'tactical' treatments on farms where high numbers of anthelmintic treatments are applied annually. In this situation it would be ideal for quarantine treatments. On farms where relatively few treatments are given annually adopting this combination strategy may be a practical and cost effective option, however, refugia then becomes a critical issue and it is vital to ensure some portion of the nematode population remains unselected.[9]

Despite the relatively high levels of resistance that were assumed for BZ and LEV in this study, they still played a useful role in helping to delay the development of resistance to a new anthelmintic class and the MLs. It has been suggested that whenever a combination product is used it is advisable to leave some stock untreated.[9] However, leaving some sheep untreated is a contentious issue because the advantage of reducing selection for resistance must be weighed against possible production losses, including deaths, in untreated animals in some regions and seasons. Because of the harsh variable climate in Australia, refugia is one issue that cannot be ignored and in many situations, leaving some sheep untreated may be the only way to provide a reliable source of unselected nematodes. This strategy is based on targeted treatment[15] of stock in poor condition or leaving some healthy animals untreated rather than treating the entire flock. It has been studied in the field[16-18] and investigated by computer

simulation to optimise the percentage of adult stock that may be left untreated.[13] In WA, where there is little refugia as infective larvae on pasture during the hot-dry summer period, leaving 1-4% of adults untreated throughout the year greatly helped delay selection for resistance without compromising worm control. However, this was not the case in VIC[13] where the question of whether it was better to give the new high efficacy drug as the first or second summer anthelmintic treatment was tested and results indicated this was not an important issue (data not shown).

The simple single score developed here is an attempt to find a balance between providing effective nematode control and selecting for drug resistance. The weights used to determine the score were provided by expert opinion and will vary from place to place and over time, but this can be accommodated in spreadsheet computations as updated scores can be used as required. The final score encompasses multiple nematode species, short- and long-term nematode burdens and death rates in ewes and lambs, and development of resistance to MPL, ML and BZLV. As such, it contains a component for sustainability, which will be valued differently by producers. The score was also presented in Tables 4-6 as percentage effectiveness to control nematodes and delay resistance so that the contribution of these components to the total score can be easily assessed. Some graziers may be willing to accept some reduction in nematode control to preserve the efficacy of their anthelmintics and Tables 4-6 provide a tool to help quantify such considerations. The ability of each option to control nematodes and delay drug resistance was presented as a percentage of the worst case situation. However, if for example 'effectiveness to control worms' was expressed as a percentage of the best nematode control option, rather than the untreated control simulation, then a more conservative result would be obtained. For example, in Table 4 options MPL+COM, MPL/COM and MPL/COM/MOX had 'effectiveness to control worms' of 94%, 91% and 93%, and associated 'mean worm scores' of 40, 54 and 44, respectively, i.e. MPL/COM and MPL/COM/MOX are respectively 35% and 10% higher (worse) than the best nematode control option in this environment. Traditionally, in parasitology, efficacy of a drug or treatment regimen is expressed as a percentage improvement over untreated or negative controls, which is why the results were summarised this way. On the other hand, production studies often include uninfected control groups against which production penalties are measured. From the 'worm scores' in Tables 4-6 the loss of effectiveness from the best option can be estimated if preferred.

The schedule set out in Table 3 for NSW was simplified in terms of grazing management and minimal anthelmintic treatments to control *Haemonchus*, i.e. suited to low risk areas. For environments or farms that have a history of high *Haemonchus* risk then reliance on a schedule that includes additional grazing management and/or a drug with persistent activity such as MOX was required. The assumptions made with regard to the efficacy and persistent activity of MOX against *Haemonchus* and *Teladorsagia* (Appendix 1) lead to MOX efficacy remaining relatively high (85%+) even when ML resistance is high and some but not all ML-resistant incoming larvae are prevented from establishing in the host.

Although the simulation model and summary score are complex to describe and implement they nevertheless represent a gross oversimplification of the biological situation which involves interactions between a number of parasite species, nutrition, management practices, weather, nematode and host genetics. As such they best help design broad strategies rather than rigidly define detailed management systems for particular sites. The broad

recommendations from the model are generally consistent across the three states despite the differences in management and climate. Such findings: providing a source of refugia; using combinations; and rotating MPL with combination products can be tailored to suit the individual farmer's needs. The aim was not to be prescriptive but provide a guide to assist decisions, which are ultimately based on the objective of the farming enterprise and its resources.

The model was developed from limited climate and pasture data,[13] however, simulations were carried out using weather data from regions in WA, VIC and NSW where variations in climate and the timing of key management decisions occur. For example, some sheep producing regions of southern NSW have climate and management systems comparable to those simulated for VIC. Because of such limitations sheep producers should seek professional advice with regard to their specific sheep management and nematode control issues so that a suitable regimen can be adapted to their circumstances. It is important that all involved in the Australian wool industry work towards preserving the effective life of available anthelmintics.

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Appendix 1 Simulating drug efficacy and selection for anthelmintic resistance.

Table A1 provides the assumed efficacy for the anthelmintics against each species and genotype; worm genotypes are denoted SS, RS and RR to represent homozygote susceptible, heterozygote, and homozygote resistant genotypes, respectively. Because the model[5] can only simultaneously simulate the development of resistance in three drug groups, developing resistance was simulated for: 1. MPL; 2. ML and 3. BZ+LEV as a combination (BZLV), simulated as a single gene. For all drugs the time to resistance was the time taken for the R-allele frequency to reach 50%. This was chosen as the initial R-allele level for BZLV was assumed to be 40%.

1. Monepantel: Resistance to MPL was assumed to be co-dominant; if in future resistance is discovered to be recessive then resistance will take longer to develop than predicted by the model and if dominant, resistance will occur in a shorter time frame. However, the results are calculated as the 'relative time' to resistance and therefore the precise mode of inheritance is less important as the ratio of time to resistance is compared not the estimated time (e.g. resistance to MPL may develop three times slower under one option by comparison with another option etc.). The R-allele frequency that in part determines the rate at which resistance develops to MPL was set so that resistance to MPL would develop in 10 years or less when MPL was used exclusively. For each management system, the R-allele frequency below which resistance to MPL did not develop at all in 20 years when MPL was used exclusively was also assessed.

2. Macrocyclic lactones: For *Teladorsagia* and *Haemonchus* MOX has usually demonstrated relatively high efficacy against ML-resistant genotypes; ABA efficacy against ML-resistant genotypes is less than MOX but greater than ivermectin.[9] Because ML resistance in *Trichostrongylus* is rare it was assumed to be recessive for both ABA and MOX.

3. BZ/LEV combinations: In all simulations BZ and LEV are only given as part of a combination treatment with ABA. For BZ and LEV, the efficacies given in Table A1 were used to calculate the efficacy of a combination BZLV treatment against individual genotypes as shown in Table A2. In Table A2 genotypes are depicted by setting B, b, L, l to represent resistant (upper case) and susceptible (lower case) alleles to BZ and LEV, respectively. To determine the 'Actual Efficacy' of a combination treatment in Table A2 the proportion of genotypes removed was calculated by assuming BZ and LEV were applied sequentially.[3] For example, given 100 *Haemonchus* worms with say genotype BbLL then 50% and 10% are killed by BZ and LEV, respectively (from Table A1), if assuming no synergy then 50 worms remain after the BZ is applied, then 5 (10%) of the remainder are removed by LEV to leave 45 worms, i.e. 55% are removed. The average of the efficacy for BZ-LEV-genotypes with the total number of R-alleles <2, =2 and >2 was used to represent SS, RS and RR genotypes to BZLV in the simulations. That is, the mean efficacy given in Table A2 was used against BZLV-combination genotypes when BZLV was applied. To estimate the accuracy of this approximation BZ, LEV and ABA were simulated as separate independent genes using the efficacies from Table A1 for COM only treatments of sheep under a VIC management regimen. The same simulation was again run but with only two independent genes: one for BZLV using the 'Average Efficacy' from Table A2; and one gene for ABA as defined in Table A1. In both simulations the initial R-allele frequency was set at 40%, 40% and 3% for BZ, LEV and ABA, respectively for both worm species. Under the VIC regimen three

paddocks were used and Table A3 shows the increase in percentage R-allele frequency over 20 years on each paddock for each drug, worm and method of simulation. Results were similar for both methods with resistance developing slightly faster when BZ and LEV were simulated by a single gene than when simulated by two independent genes. Though imposing some limitations on simulated treatment options, i.e. BZ and LEV can not be applied independently but only in combination, Table A3 indicates using one gene for the BZ+LEV treatments provides a reasonable approximation to using two genes. This then allows MPL and ML resistance to each be simulated separately by different genes as the model provides a total of three genes for simulating the development of resistance.

Tables A1-A3 for Appendix

Table A1 Anthelmintic efficacy (proportion killed) against homozygote susceptible (SS), heterozygote (RS), and homozygote resistant (RR) worm genotypes.

Drug	*SS*	*RS*	*RR*
Haemonchus			
Monepantel	0.999	0.500	0.100
Benzimidazole	0.999	0.500	0.100
Levamisole	0.999	0.999	0.100
Abamectin	0.999	0.804	0.190
Moxidectin	0.999	0.999	0.873
[a]Moxidectin L3	0.95	0.55	0.55
Teladorsagia			
Monepantel	0.999	0.500	0.100
Benzimidazole	0.999	0.500	0.100
Levamisole	0.999	0.500	0.100
Abamectin	0.999	0.999	0.603
Moxidectin	0.999	0.999	0.857
[a]Moxidectin L3	0.95	0.13	0.13
Trichostrongylus			
Monepantel	0.999	0.500	0.100
Benzimidazole	0.999	0.500	0.100
Levamisole	0.999	0.500	0.100
Abamectin	0.999	0.999	0.873
Moxidectin	0.999	0.999	0.873
[a]Moxidectin L3	0.95	0.55	0.55

[a]For *Haemonchus*, *Teladorsagia* and *Trichostrongylus* persistent efficacy of moxidectin against incoming infective larvae was assumed to last for 32, 32 and 5 days respectively.

Table A2 Proportion of different worm genotypes removed by a combination treatment of BZ and LEV. Genotypes are represented by B, b, L, l to indicate resistant (capital) and susceptible (lower case) alleles to BZ and LEV, respectively.

Total number of R-Alleles	Genotype	*Haemonchus* [a]Actual efficacy	*Haemonchus* [b]Mean efficacy	*Teladorsagia/Trichostrongylus* [a]Actual efficacy	*Teladorsagia/Trichostrongylus* [b]Mean efficacy
0	bbll	0.999999		0.999999	
1	bbLl	0.999999	0.999833	0.9995	0.999666
1	Bbll	0.9995	for 'SS'	0.9995	for 'SS'
2	BbLl	0.9995		0.75	
2	BBll	0.9991	0.999233	0.9991	0.916067
2	bbLL	0.9991	for 'RS'	0.9991	for 'RS'
3	BBLl	0.9991		0.55	
3	BbLL	0.55	0.5797	0.55	0.43
4	BBLL	0.19	for 'RR'	0.19	for 'RR'

[a]The actual efficacy was calculated by applying BZ and LEV sequentially to the given genotype using the efficacies given in Table A1. [b]The mean efficacy is the average of the actual efficacies for genotypes with <2, =2 or >2 total R-alleles for BZ or LEV. The mean efficacies were used in the simulations to approximate efficacy of combined BZ and LEV when modelled as a single gene.

Table A3 Increase in percentage BZ, LEV and ABA R-allele frequency over 20 years when simulated by two methods under VIC sheep management given COM treatments only of BZ+LEV+ABA. Paddocks 1, 2 and 3 were grazed by ewes, weaner lambs and 'dry' adult sheep, respectively; ewes were set stocked whilst weaners share pasture rotation with adult sheep.

Worm species	Simulation [a]method	Paddock 1 BZ	Paddock 1 LEV	Paddock 1 ABA	Paddock 2 BZ	Paddock 2 LEV	Paddock 2 ABA	Paddock 3 BZ	Paddock 3 LEV	Paddock 3 ABA
Teladorsagia	1 gene	0.8	0.8	0.6	0.8	0.8	0.7	3.1	3.1	2.4
Teladorsagia	2 genes	0.5	0.5	0.1	0.6	0.6	0.7	2.0	2.0	2.1
Trichostrongylus	1 gene	0.6	0.6	0.2	0.0	0.0	0.0	6.0	6.0	1.6
Trichostrongylus	2 genes	0.4	0.4	0.2	0.0	0.0	0.0	4.2	4.2	1.5

[a]Resistance to BZ and LEV was simulated using either one gene for both drugs (1 BZLV gene) or two independent genes one for each drug (2 genes). The initial R-allele frequency was set at 40%, 40% and 3% for BZ, LEV and ABA respectively for both worm species. For both methods resistance to ABA was simulated with a single independent gene.